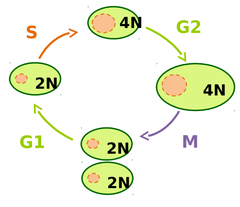
A multi-scale pipeline for reproducible analyses of tomato leaf expansion and its plasticity in response to drought

Garance Koch[1], Anaëlle Dambreville[1], Gaëlle Rolland[1], Myriam Dauzat[1], Christian Chevalier[2], Nathalie Frangne[2], Yann Guédon[3], Nadia Bertin[4], Valentina Baldazzi[4], Christine Granier[1]

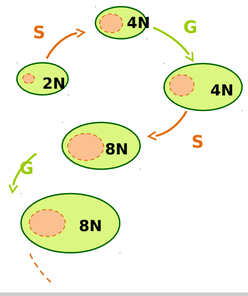
[1]INRA, Montpellier SupAgro, UMR759 Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux (LEPSE), F-34060 Montpellier, France;
[2]INRA, UMR 1332 Biologie du Fruit et Pathologie, CS20032, F-33882 Villenave d'Ornon, France;
[3]CIRAD, UMR AGAP and Inria Virtual Plants, F-34095 Montpellier, France ;
[4]INRA, Centre de recherche PACA UR 1115 Plantes et Systèmes de culture Horticoles Domaine St Paul Site Agroparc CS 40 509 - 84914 Avignon Cedex 9

Context and objectives

Mitotic cell cycle

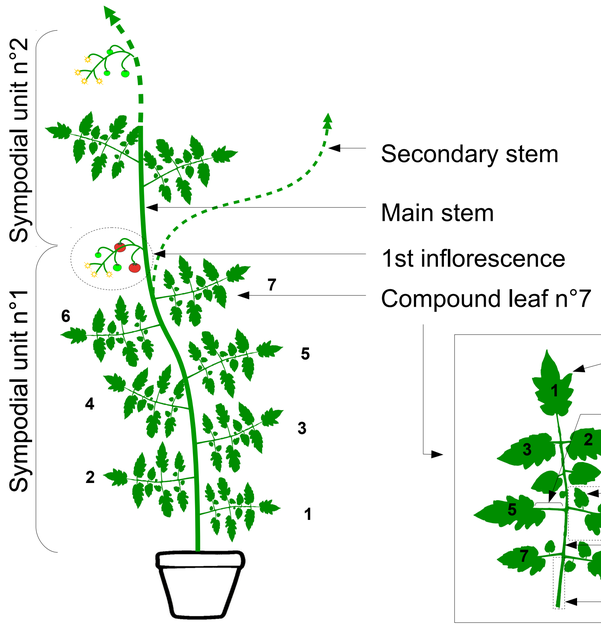


Endoreduplication cycle

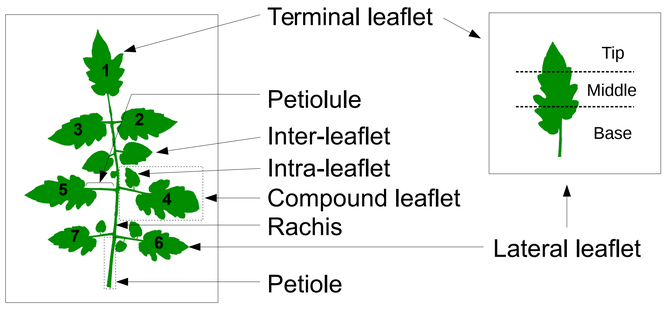


- Cell division and expansion both contribute to final leaf size in different plant species (*Gonzalez et al., 2012*). Correlations between final leaf size and cell number suggest that the cell cycle is a key process in leaf growth control. In some species, cells can also enter the specialized endoreduplication cycle. This variant of the cell cycle allows iterative DNA replication without cell division.
- The role of endoreduplication in plant functioning is not yet fully elucidated. But, it results in the increase of nuclear ploidy and influences cell growth. The functional links between cell division, cell expansion and endoreduplication during leaf development have been analysed in plants with simple leaves such as *A. thaliana* but remain unclear (*Massonnet et al., 2011*). The contribution of these 3 processes to tomato leaf expansion was studied here, taking into account the complexity of compound leaf architecture.
- Endoreduplication data are summarized as mean numbers of endocycles (expressed in percentage) : Endoreduplication Factor (EF) = (0x%2N)+(1x%4N)+(2x%8N)+(3x%16N)+(4x%32N)+...

Plant material and leaf architecture



- **Genotype** : *Solanum lycopersicum,* cv. WVA 106.
- **Multiscale leaf growth phenotyping :**
  - *Within-leaflet* : base, middle & tip
  - *Between-leaflets* : within-leaf
  - *Between-leaves* : within-sympodial unit



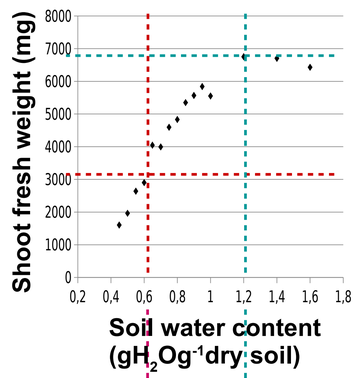
Imposing stable and reproducible soil water contents

- **Adaptation of a high-throughput phenotyping platform to grow tomato plants.** PHENOPSIS automaton (*Granier et al., 2006*) was adapted to grow plants in big pots.

Watering and imaging stations to weight, irrigate pots to target soil water content and take shoot images automatically.

The PHENOPSIS automaton developed for *A. thaliana* plant phenotyping (504 pots of 225 ml) was modified for larger plants such as canola, salad and tomato (70 pots of 7 l).

- **Shoot growth response to soil water content was established to select 'well-watered' and 'water deficit' conditions for further experiments.**



Multi-scale analysis and results

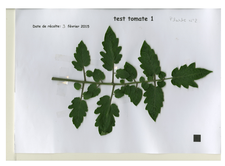
Whole leaf and leaflet masses and areas

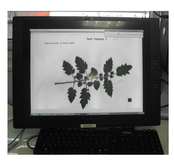

Fresh weight


Harvest in bag


Dry mass after 3 days at 60°C


Scanner


Leaf scan .jpg

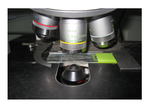

Leaf and leaflet areas

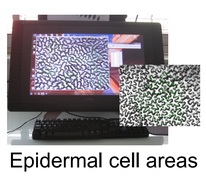
Analyses with ImageJ software on WACOM tablet

Leaflet epidermal cell areas


Translucent varnish coat


Imprint


Microscope coupled to a camera to take images


Epidermal cell areas

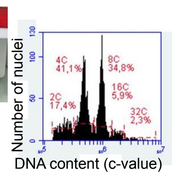
Leaflet Endoreduplication factor

Punch : Peace of leaf

Freezing : N2 (-196°C)


Nuclei extraction and coloration

C6 BD Accuri Cytometer

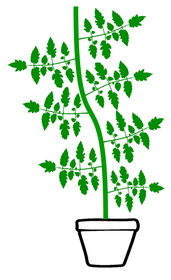


Ploidy level

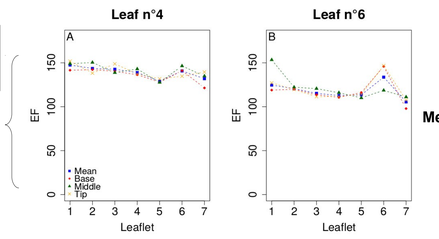
Within-leaflet scale of analysis

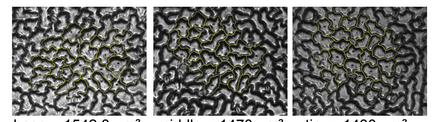

.txt and .csv datasets listed and sorted in a spreadsheet

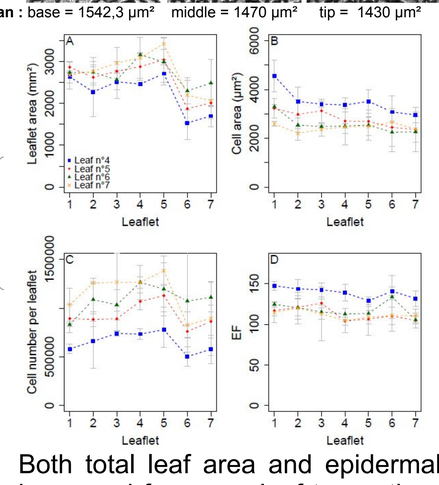
Between-leaves scale of analysis

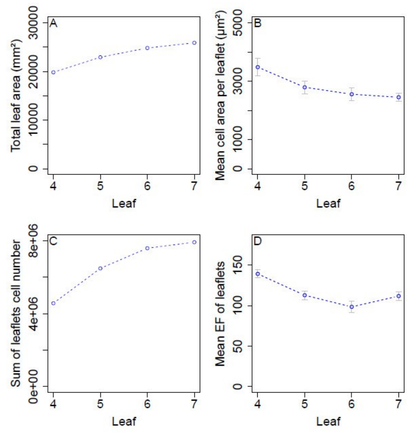


Between-leaflets scale of analysis




Mean : base = 1542,3 µm² middle = 1470 µm² tip = 1430 µm²





No variability of endoreduplication factor (EF) and cell area from the base to the tip of the leaflet.

Epidermal cell area, epidermal cell number and endoreduplication factor (EF) did not differ significantly from one leaflet to another across a same leaf as shown for leaves 4, 5, 6, and 7.

Both total leaf area and epidermal cell number per leaf increased from one leaf to another along the shoot (from leaf 4 to leaf 7). In contrast, epidermal cell area and the endoreduplication factor decreased from leaf 4 to 7.

Conclusions

- Compound leaves are comparable to simple leaves at the cellular scale.
- Gradients in endoreduplication and cell area oberved here among successive leaves are comparable to those reported for other plants such as *A. thaliana.*
- Final leaf size is related to epidermal cell number.

Model project

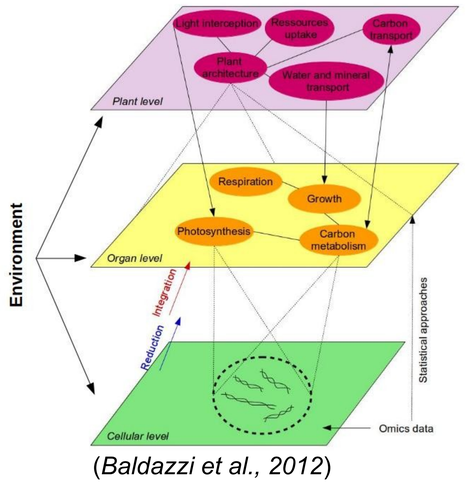
A predictive model integrating the main processes of organ growth has been developed for tomato fruit (*Baldazzi et al., 2012*)

Objective

Test this generic model to predict interactions among the main processes controlling the development of source (leaf) and sink (fruit) organs in tomato

Experiments

- Ecophysiological measurements
  - Plant status :
    - Hydric potential
    - Photosynthesis
    - Fruit and leaf set
    - Fruit and leaf microclimate
  - Dynamic of fruit and leaf growth
  - Transpiration
  - Fruit and leaf cells
    - Cell number
    - Cell size
    - Endoreduplication level



(*Baldazzi et al., 2012*)

Modeling

- Multi-scale model
  - Process based
  - Mechanistic
- Use the model to test hypothesis about
  - Processes interactions
  - Effects of environmental stresses
  - Coupling among organizational levels
- Perform *in silico* expreriments

Perspectives

- Design of multi-scale pipeline for reproducible analyses of tomato leaf expansion and its plasticity in response to drought.
- Study the impact of endoreduplication on leaf growth in tomato genotype *Solanum lycopersicum,* cv. WVA 106 and transgenic derivative lines affected in endoreduplication process.

Bibliography

Baldazzi V., Bertin N., de Jong H., Génard M. 2012.Towards multiscale plant models: integrating cellular networks. *Trends in Plant Science*,*17*, 728-36.

Gonzalez N., Vanhaeren H., Inzé D. 2012. Leaf size control: complex coordination of cell division and expansion. *Trends in Plant Science*,*17*(6),332–340.

Granier, C., Aguirrezabal, L., Chenu, K., Cookson, S. J., Dauzat, M., Hamard, P., Simonneau, T. 2006. PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water de cit in *Arabidopsis thaliana* permitted the identification of an accession with low sensitivity to soil water deficit. *New Phytologist*, *169*, 623–635.

Massonnet C., Tisne S., Radziejwoski A., Vile D., De Veylder L., Dauzat M., Granier C. 2011. New insights into the control of endoreduplication: endoreduplication could be driven by organ growth in *Arabidopsis* Leaves. *Plant Physiology*, *157*(4), 2044–2055.